Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 12/6/2019 6:56:02 PM
To: Jerry Campbell [JCampbell@ramboll.com]; Harvey Clewell [HClewell@ramboll.com]
CC: Robinan Gentry [rgentry@ramboll.com]; Walsh, Patrick [patrick-walsh@denka-pe.com]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Jones, Samantha [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=eac77fe3b20c4667b8c534c90c15a830-Jones, Samantha]; Lavoie, Emma [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=86ac7844f12646c095e4e9093a941623-Lavoie, Emma]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Kirby, Kevin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=cbb65672f6f34545be460a66ff6fa969-Kirby, Kevin]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; White, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4e179825823c44ebbb07a9704e1e5d16-White, Paul]; Hawkins, Belinda [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=075561d171e845828ec67a945663a8e6-Hawkins, Belinda]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]
Subject: RE: Chloroprene PBPK: in vitro data / parameters

Warning: even heavier "weeds"! ☺

As I look at this level of detail, it raises the question, what precisely happens when a gas sample is taken, and how should the removal of CP by the sampling be handled in the model?

Clearly ~ 400 uL are withdrawn, but the current simulations assume this is instantly replaced with 400 uL clean air, resulting in a small, instantaneous decrement in concentration. Does the sampler start by injecting 400 uL clean air? In that case the current model would be correct.

But if the syringe plunger is down when inserted and it just withdraws gas, the immediate effect is to reduce the pressure in the vial, which might cause the septum to deflect inward a bit, reducing the airspace, such that there isn't an immediate drop in concentration of the remaining air. Over time air could seep in (through the puncture) until the pressure is equalized. The effect would be a gradual dilution of the remaining gas, not a sudden dilution, in which case the effect of sampling might be better described as a continuous process.

I think this is how the model was initially set up, but I thought it better to account for the episodic pattern.

For most of these data, it probably doesn't matter: the loss is small vs. metabolism. For the human lung data, I think it matters, and I'm now thinking that the best way to capture the overall trend without worrying about the exact timing may be to go back to a continual pseudo-loss term to account for the sampling. It's the average rate of loss over time in the incubation that matters.

Separately, I did look at the control data plot from Himmelstein et al. (2004). The resolution of the captured image is low, but the loss rate predicted by the current average RLOSS looks correct. It would be better if those original data were also available. (And ultimately the EPA may need to provide all data we can, like the control data spreadsheet, in the science inventory).

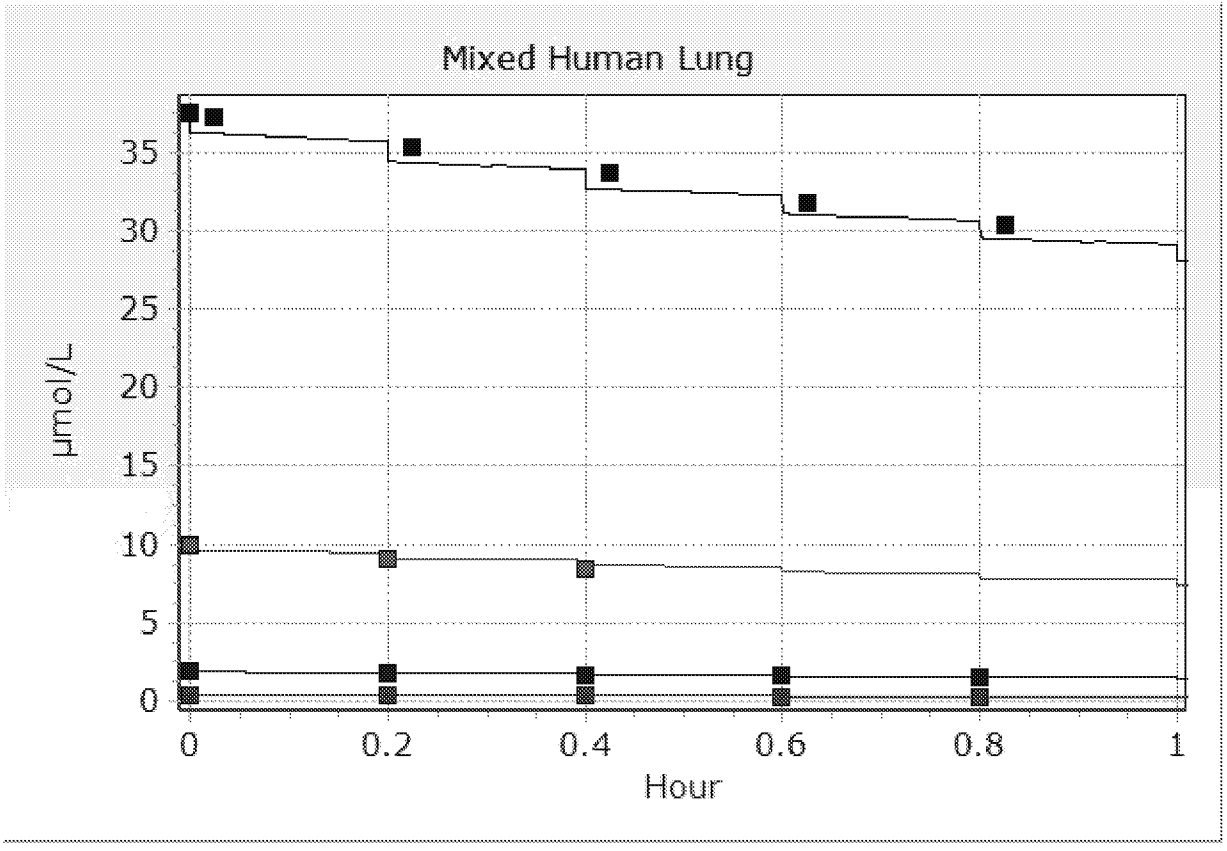
-Paul

From: Schlosser, Paul
Sent: Thursday, December 05, 2019 3:35 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>; cvanlandingham@ramboll.com
Subject: RE: Chloroprene PBPK: in vitro data / parameters

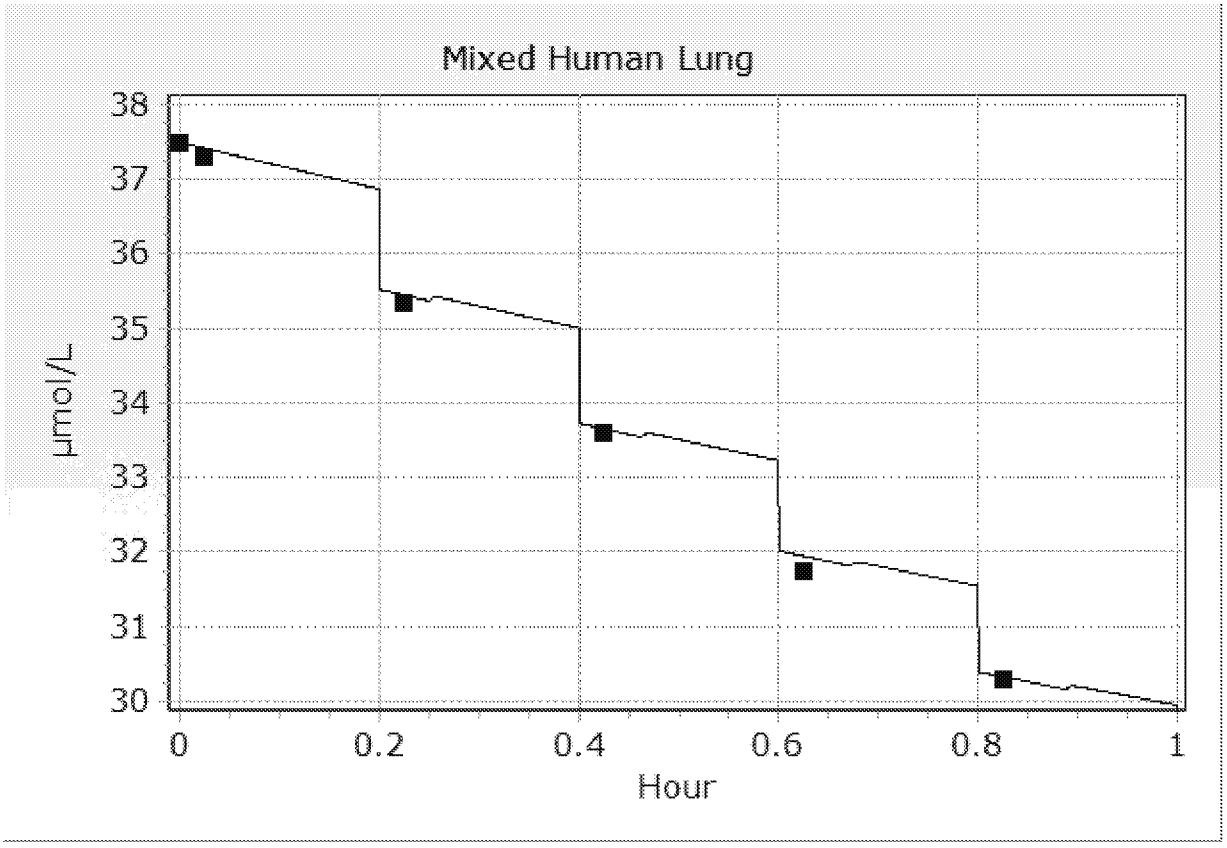
Jerry, all,

Sorry if we've been through this before and I've forgotten. But why is VINJ (sampler volume) for the human lung script (both plotting and the MCMC analysis) set to 0.0004, when all the other experiments from the 2004 paper use 0.0003858? Given the minimal metabolism in this tissue, the small difference matters and it stands out that this is the one tissue where the value is rounded off.

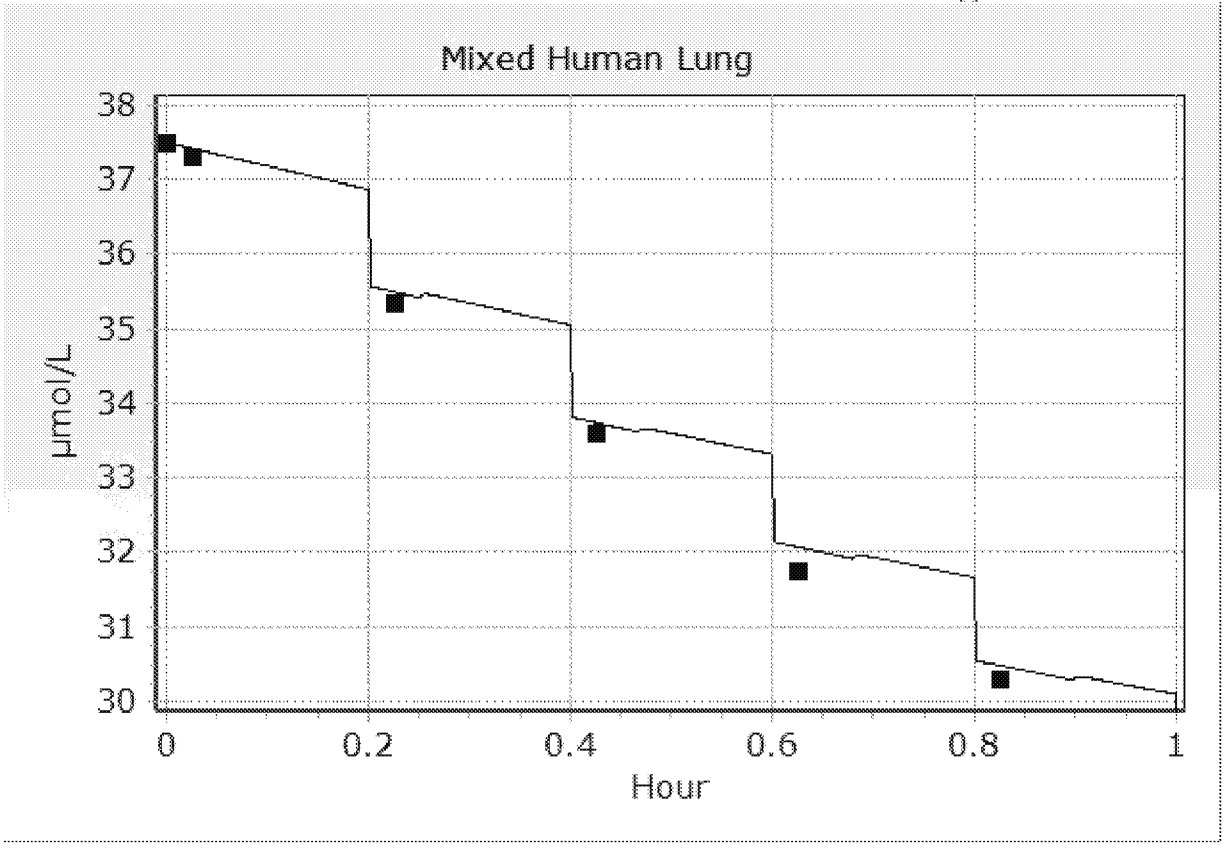
Before showing the impact, there's also a technical issue that I think needs to be addressed: when the first sample is assumed to occur. The first plot below has the model as coded, with the first sample occurring at time=0. That's when it happened, but one presumes that the concentration measured is the concentration in the vial *after* the sample is taken. The first plot below is with the current simulated sample timing, highest concentration data for the human lung.



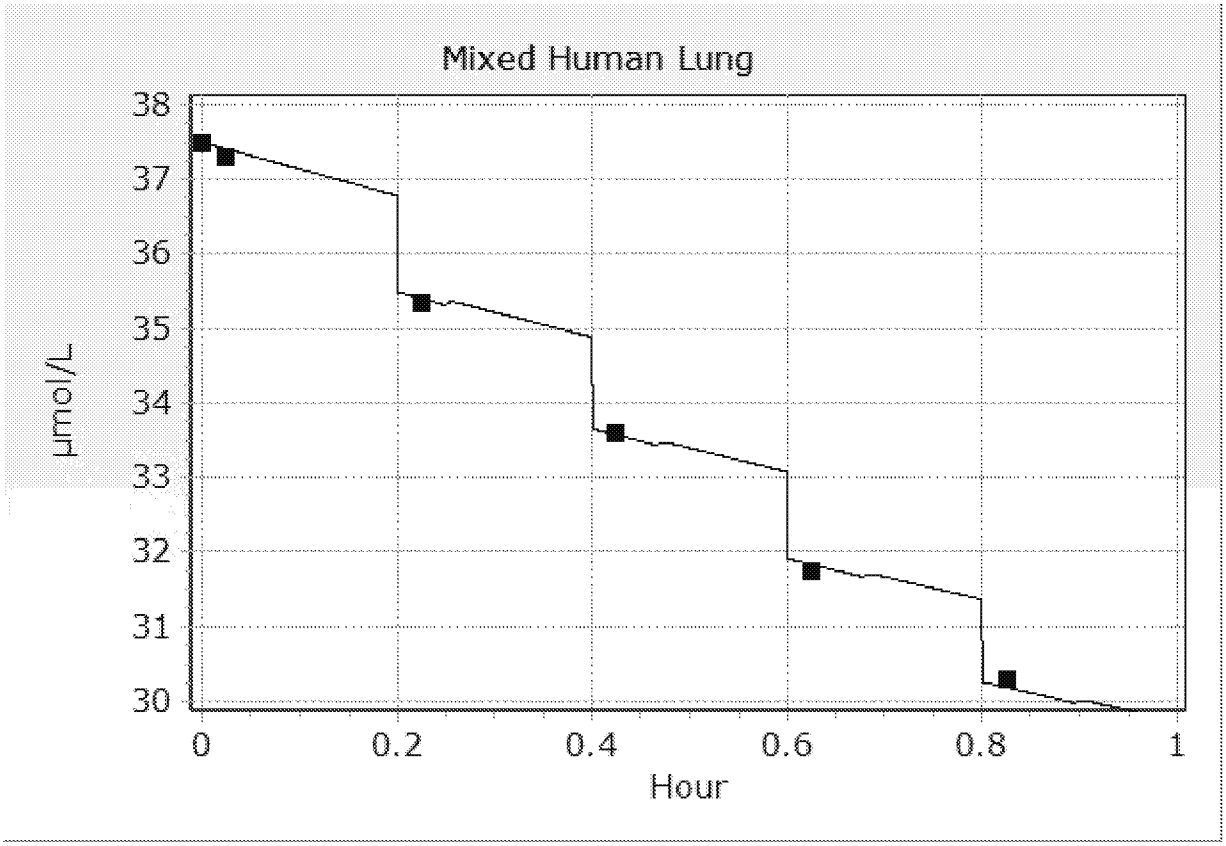
First, for others reviewing this, the "stair step" pattern of the model is due to the fact that the model includes the removal of ~ 400 uL samples every 12 min. The model accounts for the mass of CP thereby removed with each sample. More on this below.
Now the trend shown above is about right, but given that the initial condition is effectively too low, of course the attempt to fit these data will drive the metabolism estimate towards zero. In the next simulation I've moved the timing of the first sample to the first nominal sample time, 0.2. (It's possible to make the two line up exactly, but the coding of that is a bit more tricky.)



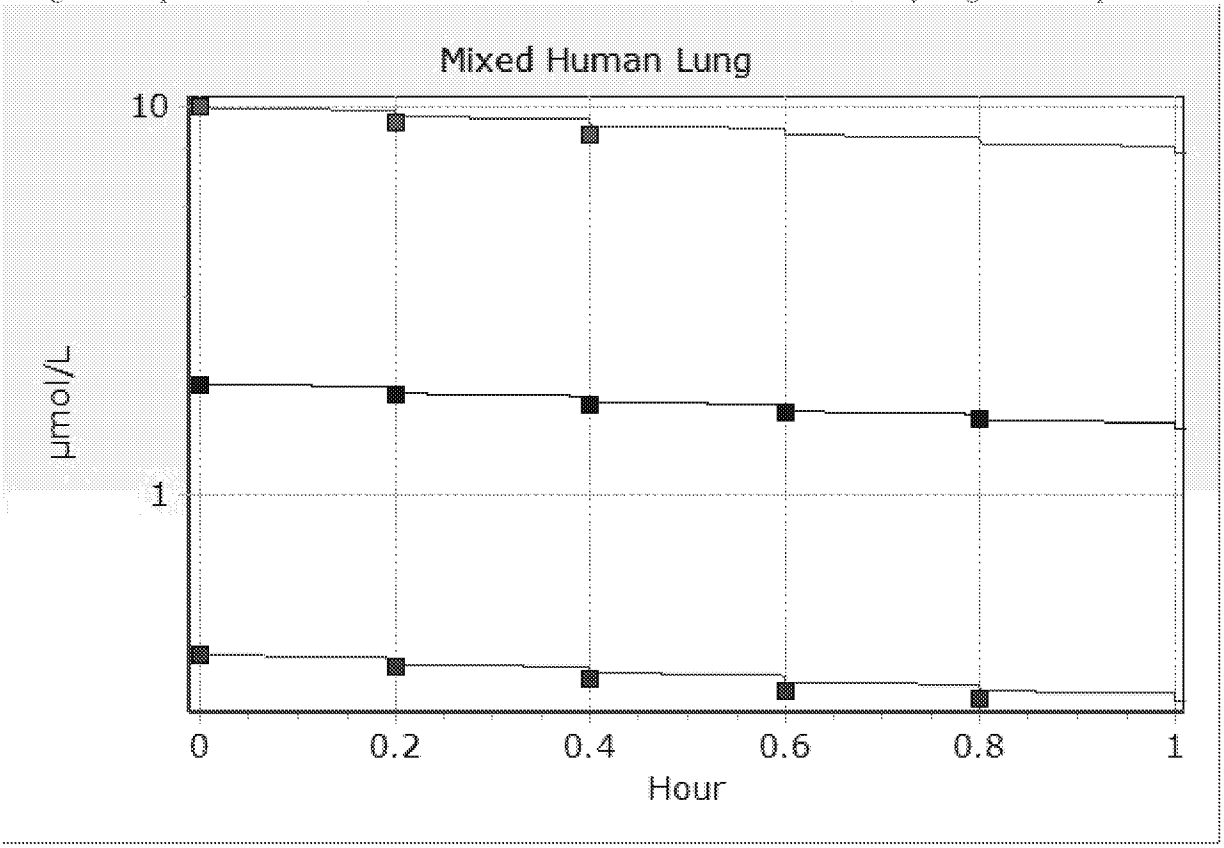
Now the simulation matches the data much more closely (I've changed the scale to show this better), and it certainly looks like there's almost no contribution due to metabolism. But that is with VINJ = 0.0004. Now what happens with VINJ= 0.0003858?



If you look at the last data point in particular, the simulation is now above the point, not through it. That's with metabolic constant KF = 2.73e-14. To get a pretty good average fit to these data (and I'm just looking at the high conc data right now), I have to increase KF to 2e-4, almost 10 orders of magnitude:



Using that sample volume and KF, here are the results for the lower three doses; if anything a bit over-predicted:



The model is somewhat above the data, indicating that in fact metabolism is a bit under-predicted still.

The other thing I am wondering a bit is the fact that the first two time points for the highest concentration (black squares) are almost at the same concentration, do not differ as if another 400 uL sample were taken. Is this b/c the 2nd point is from an incubation vial where the sample was delayed, or does this suggest that the sampling actually does not remove so much CP?

Either way, I think that the use of a different VINJ here, and the model-code-timing of the effect of that sample, is creating a bias in the analysis of the human lung data, so it's under-predicted. I know the report goes on to suggest a larger value based on a CYP marker activity. But I think the MCMC analysis of these lung data should be repeated using the same VINJ as the other 2004 data, and with simple change in the code to schedule the first sample "step" at "TI", not TS=TF=0.

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Wednesday, December 04, 2019 1:29 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Robinan Gentry <rgentry@ramboll.com>; Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>; cvanlandingham@ramboll.com
Subject: RE: Chloroprene PBPK: in vitro data / parameters

Paul,

Can you redo the figures below not adjusting for protein level? I think that will take care of some of your concern below.

Jerry Campbell

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, December 4, 2019 12:51 PM
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Cc: Robinan Gentry <rgentry@ramboll.com>; Walsh, Patrick <patrick-walsh@denka-pe.com>; Thayer, Kris <thayer.kris@epa.gov>; Jones, Samantha <Jones.Samantha@epa.gov>; Lavoie, Emma <Lavoie.Emma@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Kirby, Kevin <KIRBY.KEVIN@EPA.GOV>; Vandenberg, John <Vandenberg.John@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; White, Paul <White.Paul@epa.gov>; Hawkins, Belinda <Hawkins.Belinda@epa.gov>; Cynthia Van Landingham <cvanlandingham@ramboll.com>
Subject: RE: Chloroprene PBPK: in vitro data / parameters

Jerry,

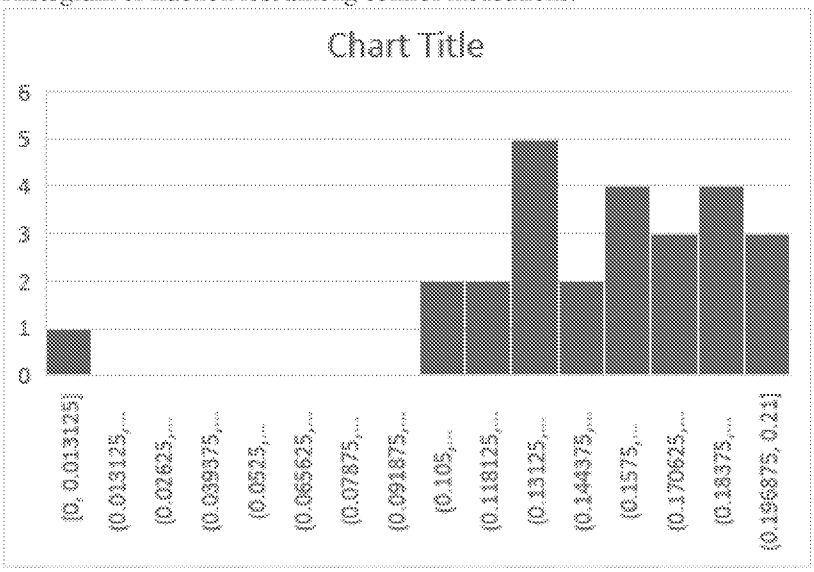
The posterior distribution was fixed (see previous email) and based on a posterior chain that encompassed 8 experiments. Since all experiments were run at the same time, the background across all vials (not individual experiments within the total experiment) would be the most accurate distribution of loss. You cannot treat a single vial/vial experiment as a matched control.

I agree that they should not be matched one-for-one.

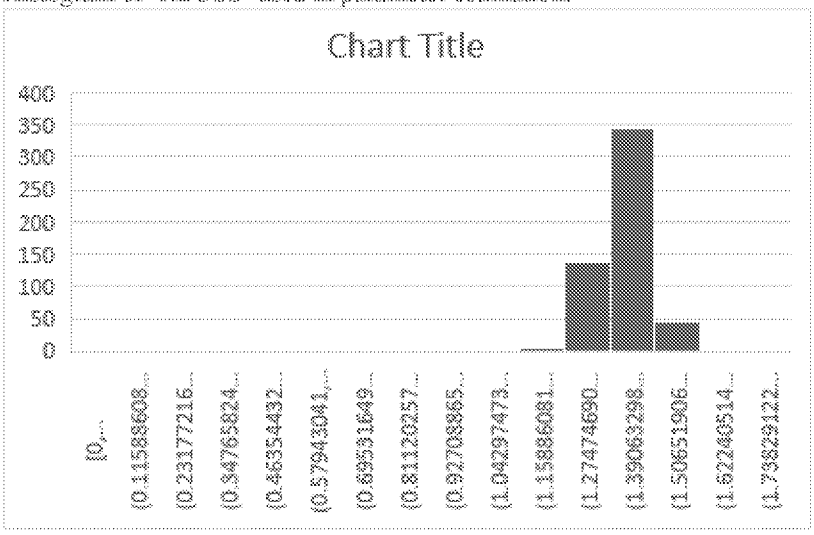
That's not how variability in an experiment like this works. The posterior chain of background loss that was sampled encompassed the totality of uncertainty in the overall experiment background loss.

This is where I disagree. Some vials only lost 11-13% in an hour, some lost 18-21%. The distribution of losses (histogram I sent) among the control experiments is not normal or log-normal. The posterior chain may represent the uncertainty in the *mean* loss rate. Below is the histogram for losses among the 35 experiments, with a '0' thrown in for scale. 2nd below is the histogram of the 'ControlData.m' values (exponentiated, x1000), with a zero and a higher point so relative to plot scale the average is the same as the histogram of the data. The data indicate that the losses from any given vial could be between 11 and 20%, with about equal probability. That is the uncertainty in the loss rate for a given active metabolism vial. The distribution used for this parameter while evaluating metabolic parameters is significantly less. It's not surprising that the uncertainty in the mean is less than the overall uncertainty. But the analysis doesn't account for the vial-vial variability.

Histogram of fraction lost among control incubations:



Histogram of 'RLOSS' used in parameter estimation:



Coupling this with the experimental data with metabolism is the best approach to incorporate RLOSS into the MCMC. This is also why the simple Nelder-Meade approach is not acceptable as that only allowed for a fixed (mean of the posterior) to be used and, thus, does not include uncertainty in the background loss.

I wasn't advocating the simpler method, but to account for the true uncertainty in RLOSS you need a distribution that looks like the first plot above (not including the point at zero), not the 2nd.

[my previous email: A linear-y plot of the same results is 2nd below. (The little 'jags' are due to the sampling pulling out CP at each time-point.) For the highest concentration data/simulation, the amount attributed to system loss, ARLOSS, is 5.9 times the amount

attributed to metabolism, so it's the predominant pathway. That the concentration decline predicted is slightly faster than the data suggest that the actual loss rate was lower in the experiment.

A loss rate that better fits the high-concentration kidney data is 0.001 instead of 0.0014, but then the metabolic clearance at lower concentrations is more underpredicted (3rd plot below). The kidney is least important, so this probably doesn't matter much… For the female mouse lung the clearance at the highest concentration is over-predicted a bit too, but over the entire data set those results are much less sensitive to the term.

So I don't think it's worth much additional work. But it just seems odd that with all the other attention to detail, a few more control experiments weren't run. And overly precise to have a rate constant to 4 significant figures when there's probably more uncertainty than that.]

I don't think your observation here is correct. There were no metabolism vials run with all experiments. The RLOSS posterior distribution is derived from a posterior distribution that was assessed from 8 experiment which totaled 34 vials without metabolism. This was the best approach to incorporate background loss into the MCMC as there is no way to know exactly what the background loss in a specific vial with metabolism was. One can only assume that it fell within the overall background loss uncertainty of the experiments. The point behind the analysis is to incorporate all of the uncertainty into the best estimate of metabolism. Parsing data that were collected at the same time under the same conditions would not be making use of the information to define RLOSS.

My initial question/concern, still not addressed, is if the observed loss rates in 2009 adequately represent the experiments done for the 2004 paper, since the vials and sampling in the earlier experiments were different. I had been assuming the loss rate data came from the 2004 paper/data, so I get that these are from 2009. But then was the loss rate the same in earlier studies? I will do some comparisons…

I agree that one shouldn't use loss-rates for a given species/sex/tissue/concentration, but instead draw from the observed distribution. But to properly capture the uncertainty in RLOSS, hence in the rate of metabolism, that distribution needs to include the between-vial variability. The posterior distribution for the mean RLOSS, what I think is being used here, does not appear to adequately represent that uncertainty.

-Paul